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Cave physical attributes influencing the structure of terrestrial invertebrate communities in Neotropics

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Abstract

The stability of temperature and humidity in caves is well known. However, little is known if higher or lower cave environmental stability (temperature, humidity, light and others) implies changes in the structure of the biological communities. Number, position and size of entrances, then size, depth, host rock and extent of the cave, the amount and type of food resources are all factors that can have strong influence on the cave biological communities. The objective of the present study was to evaluate the correlation between the presence of water bodies, size of entrances and the linear development of caves with the terrestrial invertebrate richness and species composition in 55 limestone caves located in the Brazilian Savannah, sampled from 2000 to 2011. Invertebrates were sampled by active search throughout the caves, prioritizing micro-habitats (sites under rocks) and organic resources (litter, twigs, feces and bat guano). We recorded 1,451 invertebrate species. Species richness was positively correlated with presence of cave streams, width of entrances and linear development of the caves. The richness of troglomorphic species was positively correlated to the presence of perennial pools and linear development of the caves. The presence of cave streams was a decisive factor for determining the community structure, increasing the number and the similarity of troglophile species among the caves. Flood pulses can cause disturbances that eventually select the same species besides importing resources. However, for the terrestrial troglomorphic species the disturbance caused by cave streams may decrease the number of species.

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Keywords
Cave entrances, linear development, cave streams, puddles, subterranean fauna, invertebrates

Introduction

Caves are usually dark, have constant temperature and high humidity according to the isolation from the surface, thus resulting in high environmental stability (Poulson and White 1969, Culver 1982). The availability of food resources in caves is limited, predominantly with allochthonous organic matter being imported by lotic and percolating water, bats and plant roots (Poulson and White 1969, Simon et al. 2003, Culver and Pipan 2009, Souza-Silva et al. 2011a and 2012).

Subterranean environmental stability is directly related to how isolated it is from the epigean environment. The number, width, position and distribution of the entrances in relation to the extension of the caves can increase or reduce the environmental stability of the cavity and consequently provoke changes in their biological community structures. Besides influencing environmental stability, these metrics can limit or increase the availability of food resources and likewise influence the number of species colonizing the environment (Ferreira 2004, Souza-Silva et al. 2012a).

Hydrological changes can be another factor that influences the cave fauna. Cave streams and perennial pools can act increasing the humidity and importing organic matter, being determinant for the food resources availability (Souza-Silva et al. 2012 and 2012a).

Differences in species number between distinct places have puzzled naturalists and ecologists and several hypotheses have been developed to explain these differences (Williams 1964, MacArthur and Wilson 1967). In general, species number change according to the temporal and spatial habitats traits.

It is known that the number of troglobitic species increases as the sampled area increases (Culver et al. 1999), as well as the total number of species increases with the size of cave (Culver et al. 2004, Ferreira 2004, Souza-Silva 2008). The cave size also influences the number of species at different levels according to the cave lithology. For example, the increase on the number of species as the cave size increased is more intense in iron core caves when compared to the other lithologies (Souza-Silva et al. 2011b).

Changes in species composition and richness through replacement, loss or gain among different caves of the same area or in the same cave can occur over time and space (Bento 2011, Souza-Silva et al. 2011b, Souza-Silva et al 2012a). Some methods have been proposed to evaluate beta diversity, that consider the degree of change in community composition or the community differentiation, in relation to a gradient of environment, or distinct samples (Whittaker 1960, Baselga 2007, 2010, Carvalho et al. 2012, 2013).

In this paper we verified the influences of cave metrics (width of entrances and linear development) and the presence of water bodies (presence of temporary or perennial puddles and of streams and seasonal flooding) on invertebrate cave fauna of the Neotropical region.

Methods

Study area

We conducted the study from 2000 to 2011 in 55 limestone caves of the Brazilian Savannah, northwest of Minas Gerais state, Brazil (Figure 1). According to climatic classification of Köppen-Geiger, the region is classified as *Aw* with two distinct seasons, rainy and dry. The region presents an average annual temperature between 20 and 26 °C and maximum relative humidity around 80% and minimum around 50% (Alvares 2014).

Cave metrics and water bodies

We measured the width of entrances and linear development of the caves. We considered the width of entrance as the greatest horizontal length of the entrance profile and cave linear development as the linear development sampled in each cave. Some caves were not sampled throughout their total length. We placed the caves in four categories regarding water bodies: permanently dry, with puddles (perennial or seasonal), dry but subject to seasonal flooding, and with perennial cave streams.

Sampling terrestrial cave fauna

Only terrestrial invertebrates were sampled during the study and each cave was visited once. We carried out the sampling by visual searching across the accessible parts of the cave, prioritizing organic deposits (debris, carcasses, guano, etc.) and microhabitats (spaces under rocks, humid soil, cracks, speleothems). Extensive visual searching and manual collections were made with the aid of tweezers, brushes and entomological nets (Ferreira 2004, Souza-Silva et al. 2011b). The collection team was always composed by five biologists (always the same team) with experience in caving and manual collection of invertebrates, as recommended by Weinstein and Slaney (1995). To ensure that the sample was the most standardized as possible, the sampling time was approximately 10 minutes in 10 m^2 for each biologist (Souza-Silva et al 2011b).

We separated all specimens into morphospecies taxa for all statistical analysis (Oliver and Beattie 1996, Derraik et al. 2002, Ward and Stanley 2004, Derraik et al. 2010, Souza-Silva et al. 2011b). Oliver and Beattie (1996) showed that morphospecies identified by non-specialists can provide estimates of richness and turnover consistent with those generated using species identified by taxonomic specialists. The use of morphospecies or corrected morphospecies inventories in the analyses provided results generally concordant with conventional species inventories (Oliver and Beattie 1996a).

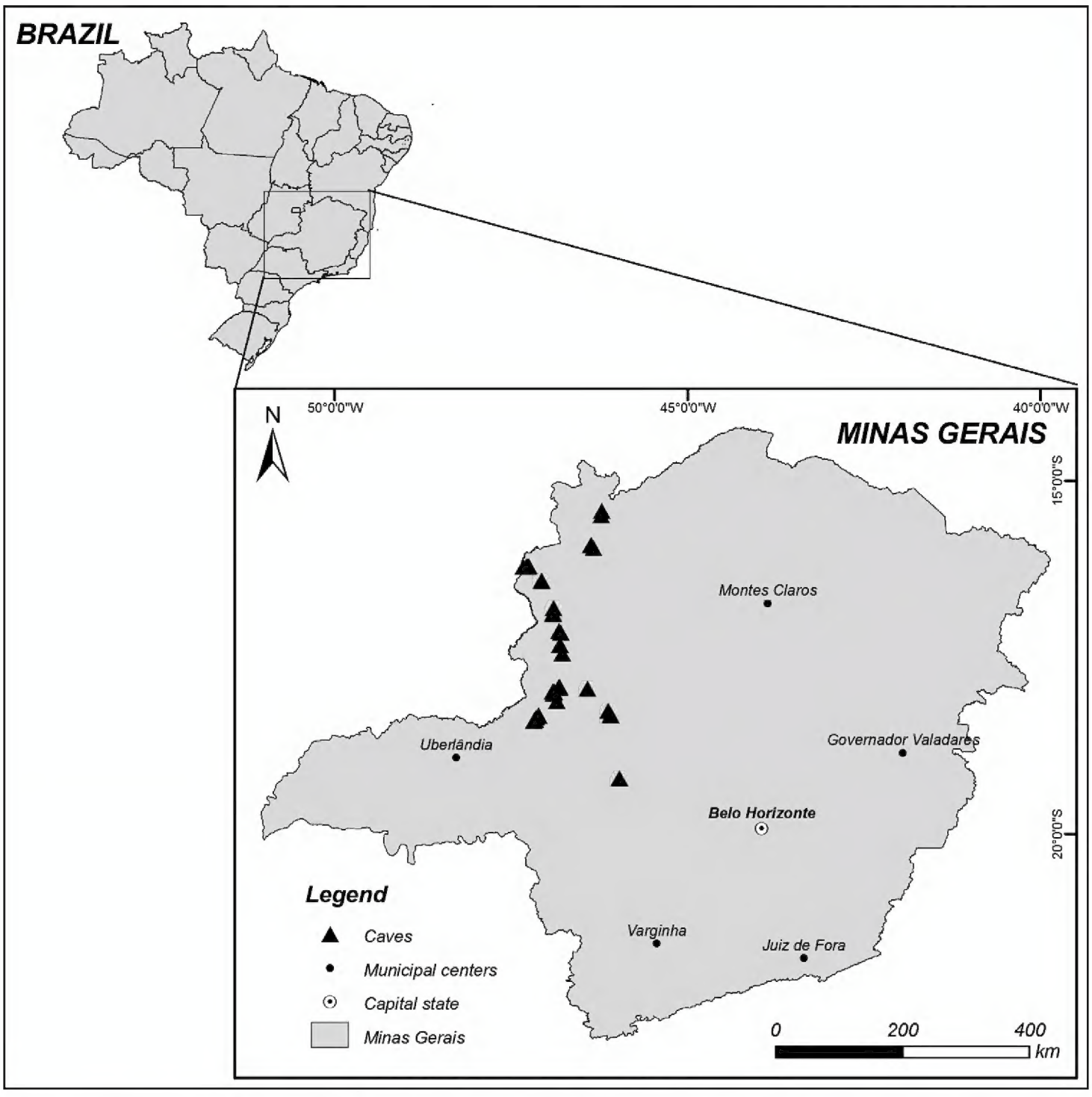

Figure 1. Cave distribution at Minas Gerais state, Brazil (black triangle), where terrestrial invertebrates were sampled.

Determination of troglobite/troglomorphic species

We determined the troglobite/troglomorphic species through the identification of troglomorphisms in the specimens. Such characteristics vary among the groups, but frequently are represented by the reduction of melanin pigmentation, reduction of ocular structures and elongation of appendages (Culver and Wilkens 2000, Culver and Pipan 2009).

Statistical analyses

To normalize the variables the values of entrance width and linear development of the caves were log-transformed to reduce the influence of extreme values. The total species

richness, was normally distributed (Shapiro Wilk test = 0.918; p = 0.001). The richness of troglomorphic species recorded a lot of zeros, and it was not possible to reach normality for this variable.

We evaluated the influence of the entrances width and cave linear development on the species richness through linear regression. Influences of the presence/absence of different categories of water bodies were evaluated with ANOVA one-way for total richness and non-parametric ANOVA (Kruskal-Wallis test) for richness of troglomorphic species. One of the sampled caves (Deus Me Livre Cave) possesses a singular condition: despite being dry throughout the year, it is subject to seasonal flooding, and was not considered in the analyses.

We used the Jaccard index to compare the fauna composition in different caves (Magurran 2004). This index is the most suitable for presence/absence data since it does not assign weight to the species abundance, such as the Bray-Curtis index commonly used in ecological studies. Beta diversity has been calculated in accordance with the proposals of Carvalho et al. (2012, 2013) and Cardoso et al. (2015), in which it is possible to perform the partition of this measure by means of the contribution of replacement and differences in species richness. We performed the partition of beta diversity using BAT package developed by Cardoso et al. (2015). The objective of this analysis was to assess whether the dissimilarity between the communities was influenced more by replacement than by differences in species richness.

We performed the DistLM test to verify the influence of metric parameters and the presence/absence of different categories of water bodies on species composition of the caves (Anderson 2004). This test shows which variable or variables can influence the fauna composition (McArdle and Anderson 2001). We used non-metric multidimensional scaling (nMDS), based on the Jaccard index, to visualize groups of caves according to the variables that best explained the species composition identified in the DistLM test. We performed the ANOSIM one-way (Jaccard index) analysis to test the significance of the separation of groups (Clarke 1993).

Results

The higher entrance width was recorded for Marcela Cave (125 m; Table 1). Lapa Nova Cave, with 4,000 meters sampled, presented the longest linear development (Table 1). Most of the caves (n = 38) were dry throughout the year and others had water bodies. Nine caves had puddles, one was subject to seasonal flooding and seven of them had rivers (Table 1).

We recorded 1,451 invertebrate taxa, distributed in at least 174 families (Table 2). Diptera presented the highest richness (326 taxa), followed by Coleoptera (250 taxa) and Araneae (169 taxa) (Figure 2). Families with the highest number of taxa recorded were Chironomidae (45taxa), Staphylinidae (79) and Theridiidae (24) respectively (Table 2). The average richness was 58 morfospecies (SD = 26). Lapa Nova cave presented the highest richness (153 taxa), followed by the Vereda da Palha cave (107 taxa) (Table 1).

Table 1. Municipalities, caves, water bodies (WB) (CS: cave streams, P: puddles, SF: dry caves subject to seasonal flooding, D: dry), width of entrances (WE), sampled linear development (LD), total number of species (S), number of troglomorphic species/troglobitic (TS) in the studied area.

Municipalities	Caves	WB	WE (m)	LD (m)	S	TS
Arinos	Camila	CS	5	120	98	2
	Capa	CS	17	480	101	0
	Marcela	CS	125	400	78	0
	Suindara	D	16.9	160	56	0
	Salobo	P	6.8	40	47	2
	Taquaril	CS	5	150	70	1
	Velho Juca	D	7.2	70	47	2
Cabeceira Grande	Caidô	D	30	400	71	1
	Porco Espinho	D	4	17	36	0
Coromandel	Huguinho	D	4	35	38	0
	Urubu	D	2	50	34	0
	João do Pó	D	4	180	48	0
	Ronan	D	10	1000	46	0
	Ronan II	D	6.5	160	25	0
	D'água	P	9	80	33	0
	Morcegos	D	3	86	31	0
João Pinheiro	Sapecado	D	1.5	20	26	0
	Tauá	D	15.4	26	22	0
Lagamar	Vendinha	D	7	300	72	0
Matutina	Cachoeira	P	13.3	20	59	0
	Nove	D	1.6	7.85	48	0
	Campo de Futebol	D	15	25	42	0
Paracatu	Lagoa Rica	P	5	200	53	6
	Tamanduá II	D	2	38	41	0
	Cava	D	3.3	38	48	0
	Santa Fé	D	21	78	30	0
	Brocotó	D	4.5	30	72	0
	Brocotó II	D	5	60	73	0
	Santo Antônio	P	13.8	67	51	0
Presidente Olegário	Caieira	D	22	200	61	0
	Juruva	CS	15	250	105	1
	Vereda da Palha	CS	14	250	107	0
Unaí	Abriguinho	D	6.5	8	34	0
	Barth Cave	D	14	160	47	1
	Cachoeira do Queimado	D	52	160	57	2
	Encosta	D	2	40	52	0
	Mata dos Paulista	CS	1.5	30	63	0
	Frangas	D	3	13	41	0
	Deus Me Livre	SF	9	50	106	0
	Rio Preto	D	4.6	38	56	2
	Malhadinha	D	5	70	98	2
	Sapezal	P	15	130	78	0

Municipalities	Caves	WB	WE (m)	LD (m)	S	TS
Vazante	Abrigo da Escarpa	D	10	4	36	0
	Escarpa	D	3	63.3	63	0
	Urtigas	D	30	369	70	2
	Urubus	D	24	61.3	93	3
	Não Cadastrada	D	2	18.4	49	1
	V01	D	2	5	15	0
	V02	D	1.5	10	38	2
	Delza	P	4	1400	46	5
	Mata Velha	P	7	160	61	0
	Guardião Severino	D	15	50	47	0
	Lapa Nova	P	45	4000	153	6
	Lapa Nova II	D	4.5	600	55	3
	Sumidouro da Vaca Morta	D	7	16.1	72	0

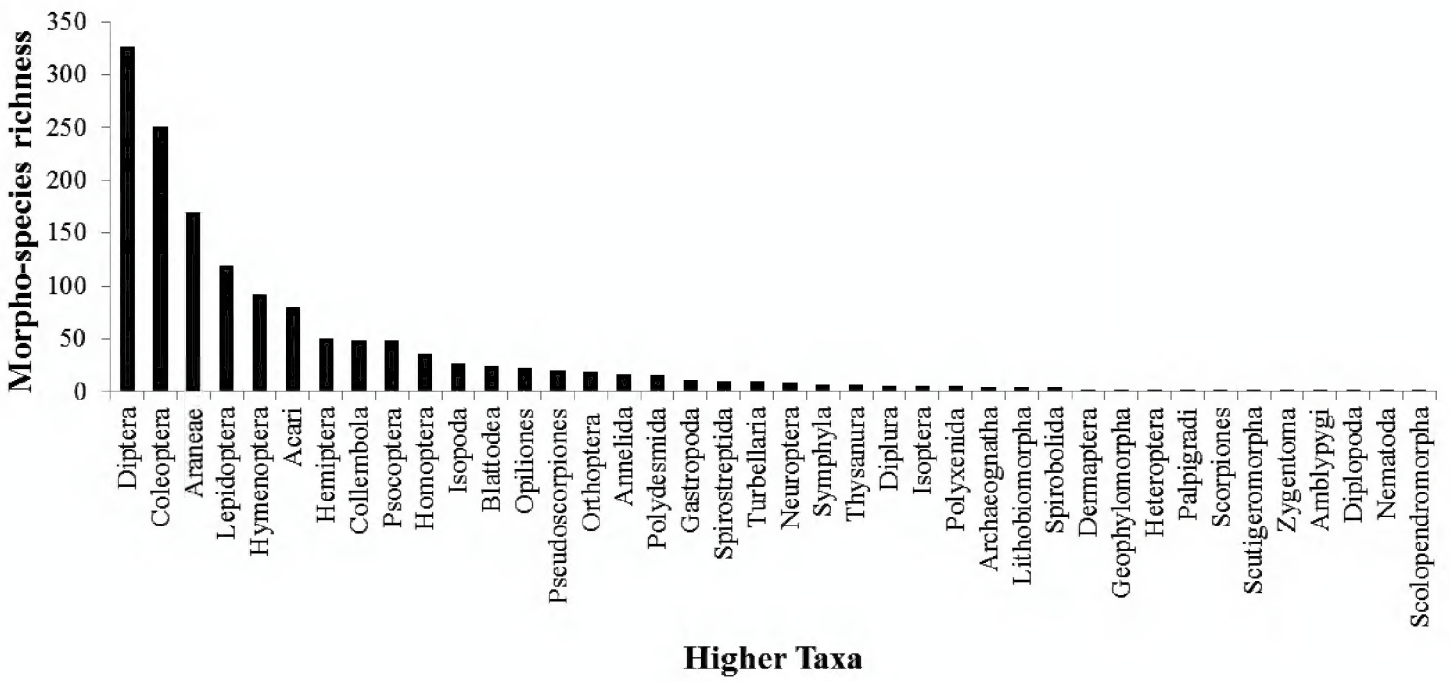

Figure 2. List of sampled higher taxa and their species richness.

Only 2.3% of the invertebrates presented troglomorphic traits (33 taxa), distributed in 18 of the 55 sampled caves. Such taxa included Araneae (eight species), Isopoda (six species), Collembola (six species), Polydesmida (five species), Acari, Hirudinea, Coleoptera, Opiliones, Palpigradi, Polyxenida, Pseudoscorpiones and Turbellaria (one species each) (Table 3). The caves with the highest richness of troglobitic species were the Lagoa Rica and Lapa Nova caves, with six species each one.

A significant difference was observed between the total richness of taxa and width of entrances (R: 0.424, p: 0.001), linear development (R: 0.519, $p < 0.001$) and presence of water bodies in the caves (R^2: 0.279, F: 9.876, $p < 0.001$), and the richness of taxa was higher in caves with rivers (Figure 3).

Table 2. Higher taxa and families recorded in 55 limestone caves in the Brazilian Savannah. Un: unidentified. Species numbers recorded for the families are inside the parentheses.

Higher taxa		**Families**
Annelida	Oligochaeta	Un
Arachnida	Acari	Ameroseiidae (1), Anoetidae (1), Anystidae (1), Argasidae (2), Bdellidae (3), Cheiletidae (1), Erythraidae (4), Ixodidae (3), Laelapidae (6), Macrochelidae (5), Macronyssidae (4), Melicharidae (1), Ologamasidae (1), Opilioacaridae (1), Otopheidomenidae (1), Parasitidae (1), Phthiracaridae (1), Podocinidae (1), Rhagidiidae (3), Teneriffidae (1), Veigaiidae (2).
	Amblypygi	Phrynidae (1)
	Araneae	Actinopodidae (1), Araneidae (16), Caponiidae (1), Ctenidae (12), Deinopidae (3), Dictynidae (1), Dipluridae (1), Filistatidae (1), Gnaphosidae (1), Leiodidae (1), Nemesiidae (2), Ochyroceratidae (2), Oonopidae (12), Palpimanidae (1), Pholcidae (7), Prodidomidae (3), Pisauridae (1), Salticidae (10), Scytodidae (2), Segestriidae (1), Sicariidae (1), Sparassidae (1), Symphytognathidae (2), Tetrablemmidae (1), Tetragnathidae (1), Theraphosidae (1), Theridiidae (24), Theridiosomatidae (2), Trechaleidae (2), Uloboridae (2)
	Opiliones	Gonyleptidae (12), Escadabiidae (2).
	Palpigradi	Eukoeneniidae (2)
	Pseudoscorpiones	Chernetidae (4), Chthoniidae (6), Garypidae (2).
	Scorpiones	Buthidae (1)
Crustacea	Isopoda	Armadillidae (2), Dubioniscidae (3), Philosciidae (2), Platyarthridae (5), Porcellionidae (4), Styloniscidae (5)
Insecta	Archaeognatha	Meinertellidae (4)
	Blattodea	Blaberidae (1), Blattellidae (15), Blattidae (8)
	Coleoptera	Bostrichidae (1), Carabidae (29), Cholevidae (3), Chrysomelidae (4), Curculionidae (6), Dermestidae (6), Dryopidae (3), Elateridae (9), Elmidae (3), Endomychidae (1), Histeridae (3), Lampyridae (2), Nitidulidae (1), Omophronidae (1), Pselaphidae (9), Ptiliidae (3), Ptylodactilidae (6), Scarabaeidae (6), Staphylinidae (79), Tenebrionidae (16)
	Collembola	Arrhopalitidae (4), Dicyrtomidae (2), Hypogastruridae (1)
	Dermaptera	Labiidae (2)
	Diplura	Japygidae (1)
	Diptera	Agromyzidae (4), Anthomyzidae (1), Asilidae (2), Calliphoridae (1), Cecidomyiidae (36), Ceratopogonidae (15), Chironomidae (45), Chloropidae (1), Culicidae (2), Dixidae (1), Dolichopodidae (7), Drosophilidae (19), Empididae (1), Keroplatidae (1), Lauxaniidae (1), Milichiidae (6), Muscidae (6), Mycetophilidae (12), Phoridae (18), Psychodidae (18), Sarcophagidae (1), Sciaridae (13), Simuliidae (3), Stratiomyidae (5), Streblidae (1), Syrphidae (1), Tabanidae (1), Tipulidae (25)
	Hemiptera	Cydnidae (6), Hebridae (10), Ploiariidae (8), Reduviidae (7), Cicadellidae (17), Cixiidae (12), Thyreocoridae (1)
	Hymenoptera	Apidae (1), Braconidae (1), Eupelmidae (1), Encyrtidae (1), Evaniidae (2), Formicidae (57), Halictidae (1), Ichneumonidae (2), Mutillidae (1), Pteromalidae (2), Vespidae (2)
	Isoptera	Termitidae (3)
	Lepidoptera	Arctiidae (3), Geometridae (2), Hesperiidae (3), Noctuidae (24), Pyralidae (7), Satyridae (1), Tineidae (54)
	Neuroptera	Ascalaphidae (1), Mantispidae (1), Myrmeleontidae (5)

Higher taxa		**Families**
	Orthoptera	Gryllidae (2), Phalangopsidae (3), Tettigoniidae (1)
	Psocoptera 4	Lepidopsocidae (2), Liposcelididae (3), Psyllipsocidae (9), Ptiloneuridae (6)
	Zygentoma 4	Atelurinae (2), Lepidotrichidae (1), Lepismatidae (1), Nicoletiidae (4)
Mollusca	Gastropoda	Un
Myriapoda	Geophilomorpha 1	Geophilidae (2)
	Lithobiomorpha 1	Lithobiidae (1)
	Polydesmida 2	Chelodesmidae (1), Paradoxosomatidae (1)
	Polyxenida	Polyxenidae (5)
	Scolopendromorpha 2	Cryptopidae (1), Scolopendridae (1)
	Scutigeromorpha 1	Scutigeridae (2)
	Spirobolida 1	Rhinocricidae (1)
	Spirostreptida 1	Pseudonannolenidae (6)
	Symphyla 2	Scolopendrellidae (2), Scutigerellidae (2)
Nematoda	Nematoda	Un
Platyhelminthes	Temnocephalida	Un
Turbellaria	Turbellaria	Un

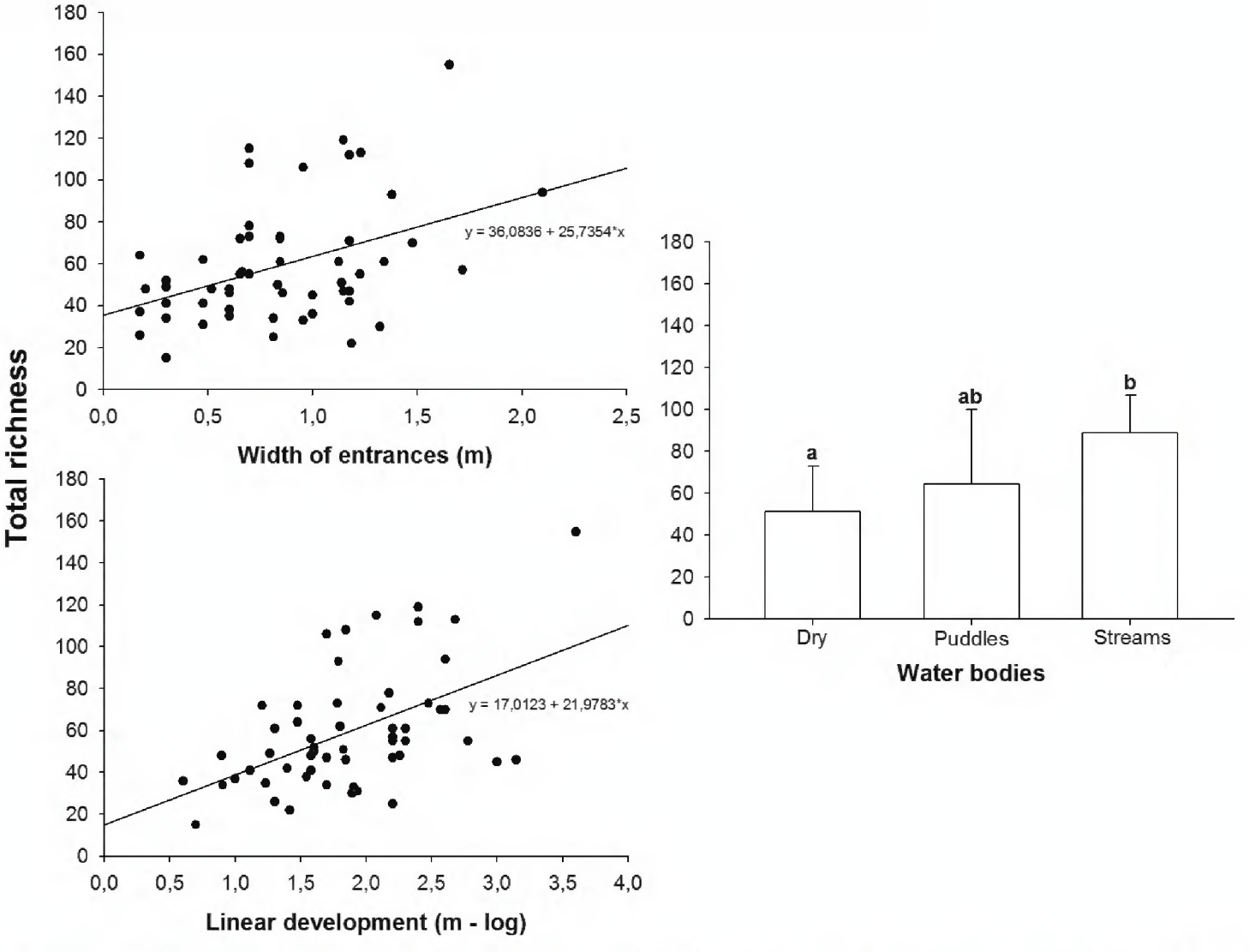

Figure 3. Correlation between total richness and width of entrances, linear development and water body presence/absence. The barr represents the average and the trace the standard deviation. Different letters indicate significant differences in average richness.

Table 3. List of troglomorphic/troglobitic species recorded in the sampled caves in the Brazilian Savannah, Minas Gerais state, Brazil, in the years 2000, 2009, 2010 and 2011. Un: unidentified.

Higher taxa	Family	Morphospecies	Caves
Acari	Un	Trombidiforme sp8	Rio Preto
Annelida	Un	Hirudinea sp3	Salobo
Araneae	Ochyroceratidae	Araneae sp24	Barth cave
		Ochyroceratidae sp1	Urubus cave
	Oonopidae	Oonopidae sp3	Lapa Nova cave
		Oonopidae sp4	Lagoa Rica cave
	Prodidomidae	Prodidomidae sp3	Cachoeira do Queimado cave
		Prodidomidae sp1	Delza cave
	Tetrablemmidae	Tetrablemmidae sp1	Lagoa Rica cave
	Un	Araneae sp17	Não Cadastrada cave
Coleoptera	Pselaphidae	Pselaphidae sp10	Rio Preto
Collembola	Arrhopalitidae	*Arrhopalites* sp1	Delza, Lapa Nova, Lapa Nova II
	Un	Collembola sp5	V02
	Hypogastruridae	*Acherontides* sp1	Lapa Nova, Lapa Nova II
	Un	Collembola sp12	Lagoa Rica
	Un	Collembola sp32	Camila
	Un	Collembola sp34	Malhadinha
Isopoda	Platyarthridae	*Trichorhina* sp1	Lagoa Rica, Urtigas, Delza, Lapa Nova
		Trichorhina sp3	Urubus
		Trichorhina sp5	Camila
		Trichorhina sp.	Velho Juca, Malhadinha
	Styloniscidae	Styloniscidae sp1	Urtigas, Delza
		Styloniscidae sp5	Juruva
Opiliones	Escadabiidae	*Spelaeoleptes* sp1	Lagoa Rica
Palpigradi	Eukoeneniidae	*Eukoenenia virgemdalapa*	Lapa Nova
Polydesmida	Un	Polydesmoidea sp1	Lapa da Delza
	Un	Polydesmoidea sp2	Lagoa Rica
	Un	Polydesmoidea sp3	Caidô, Cachoeira do Queimado
	Un	Polydesmoidea sp4	Velho Juca
	Un	Polydesmida sp2	Urubus
Polyxenida	Polyxenidae	Polyxenidae sp5	Taquaril
Pseudoscorpiones	Chthoniidae	Chthoniidae sp2	V02
Turbellaria	Un	Turbellaria sp6	Salobo

No significant relation was observed between the richness of troglomorphic species and width of entrances. However, there was a significant relation between the richness of troglomorphic species and the linear development (R: 0.460, $p < 0.001$) and presence/absence of water bodies (H: 4.722, $p < 0.013$), with higher values in caves with puddles (Figure 4).

In general the faunal troglophile composition was quite dissimilar between the caves (average B_{total}: 0.9786; variance: 0.0007). The recorded dissimilarity is explained

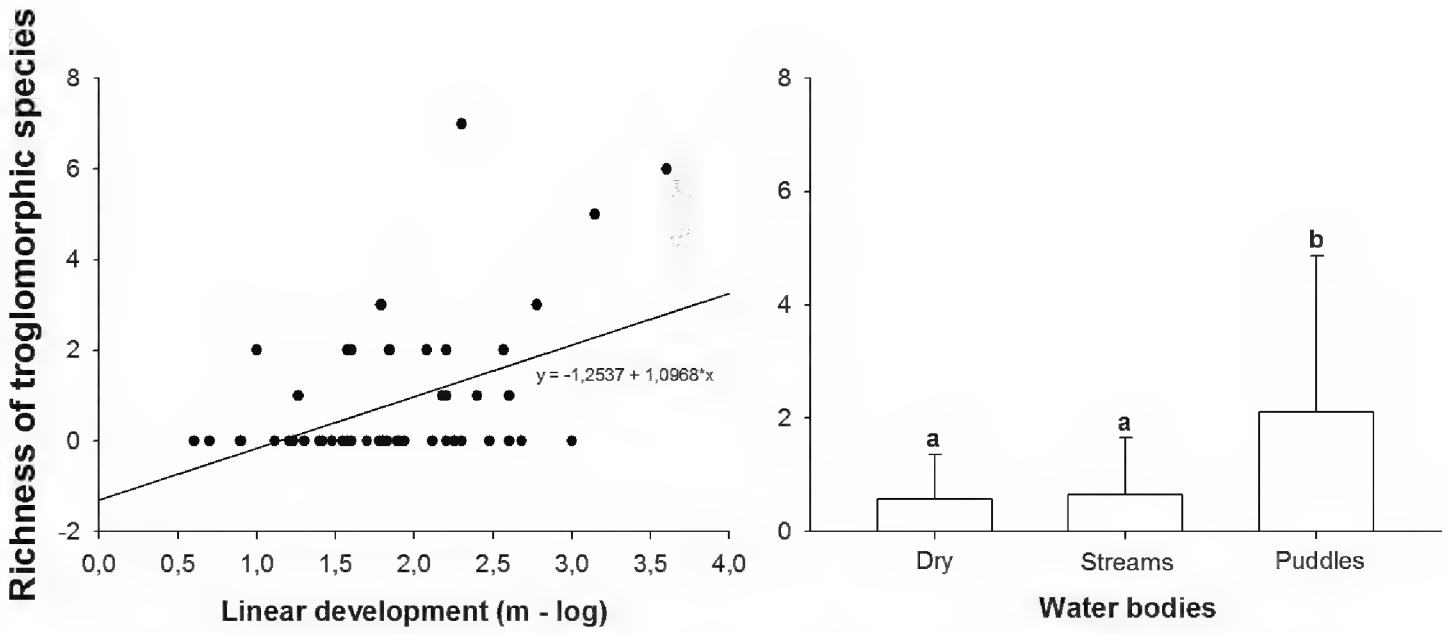

Figure 4. Correlation between the richness of troglomorphic species and linear development and water body presence/absence. The barr represents the average and the trace the standard deviation. Different letters indicate significant differences in average richness.

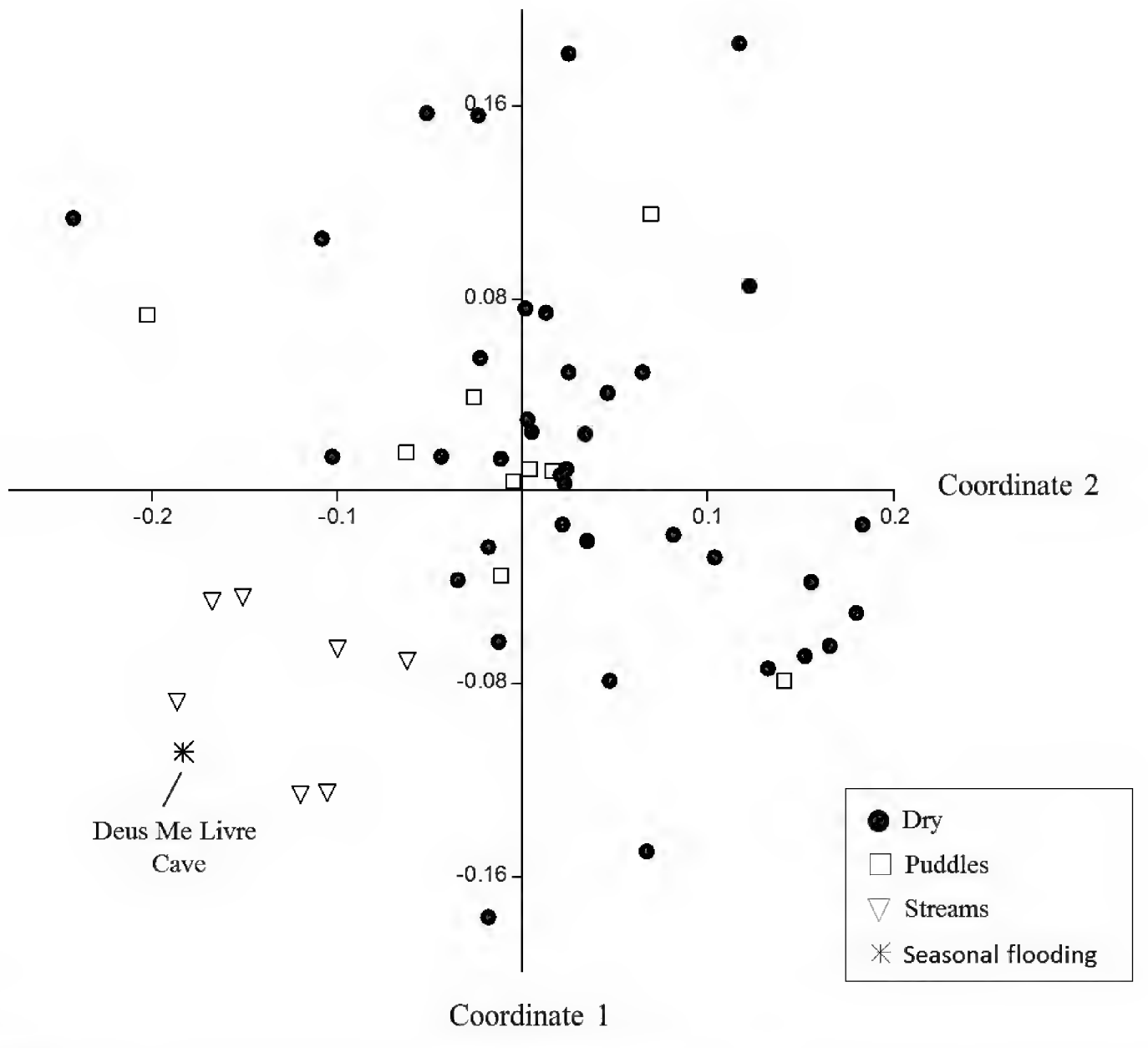

Figure 5. Non-metric multidimensional scaling (Jaccard index) using presence and absence of species sampled in 55 limestone caves of the Brazilian Savannah. The figure shows that the cave, despite dry most of the year, is subject to seasonal flooding (Deus Me Livre cave), and then was more similar to caves with streams.

by the replacement of species (B_{repl}: 0.9786705). The contribution of differences between number of species is near-zero (B_{rich} < 0.0000001).

Despite the general high dissimilarity, the presence of water bodies significantly influenced the species composition (DistLM Test, Pseudo-F: 1.901, R^2: 0.054, p < 0.001). The non-metric multidimensional scaling analysis (nMDS) showed that among the water body categories, cave with streams were more similar regarding the faunal composition (Figure 5, Stress: 0.18). This separation was confirmed by ANOSIM (one-way). A significant difference was observed between caves with streams and dry caves (R: 0.443, p < 0.001) and caves with streams and with puddles (R: 0.541, p: 0.002), while dry caves and caves with puddles were not significantly different.

Discussion

Little is known about the effects of physical characteristics determining the cave community richness and composition. Most of the studies regarding this topic showed that number of species increases in large caves and with more entrances (Culver et al. 2003, Culver et al. 2004, Ferreira 2004, Souza-Silva et al. 2011b, Souza-Silva et al. 2012). Corroborating these previous studies our results demonstrated the effects of the cave metric parameters on the number of terrestrial invertebrate species associated to limestone caves in Brazil. Regarding the influence of the presence of water bodies into the caves, our findings are new since no previous studies have shown similar results.

The relation observed between width of entrances and number of species (Figure 3) can be due to the fact that large entrances probably function as "windows" that facilitate the colonization of hypogean systems by external invertebrates as well as the input of organic matter. Caves with large entrances may have more interface areas with the surrounding epigean system, thus increasing the establishment of para-epigean communities (Ferreira and Martins 2001, Prous et al. 2004). It is worth noting that caves with more entrances potentially may be capable to receive a greater amount of organic material from the epigean environment, then increasing the food resource availability inside the caves.

It is valid to note that the tropical region presents external conditions milder than those observed in temperate climate regions. Entrances of tropical caves provide excellent shelter sites and even permanence for several species (Prous et al. 2004), different from what occurs in many temperate caves, in which the entrances, especially in the winter, are almost as severely affected by the cold as the external environment (Culver and Pipan 2009).

The increase in the linear development of caves was related to total number of taxa (Figure 3) and number of troglobitic species (Figure 4). This tendency was also observed in previous studies (Culver et al. 2003, Culver et al. 2004, Ferreira 2004, Souza-Silva et al. 2011a). Larger caves present higher habitat and resource availability, which are decisive factors for the subterranean fauna (Culver et al. 2006), thus allowing higher number of species to establish (Culver et al. 2004, Ferreira 2004, Souza-Silva 2008). As

an example, one can mention that larger caves allow the establishment of more species and larger populations of bats (Brunet and Medelin 2001), then increasing the productivity of guano. Adding our findings to the above mentioned studies we can say that there is a positive relationship among the linear development, availability and variety of habitats, resource availability and the number of species colonizing the cave environment. However, these variables work together and can influence in different ways and levels. One example is that a cave with a linear development of 200 m (Lagoa Rica) has a similar number of total species (53 species) to Delza cave, with a linear development of 1400 m (46 species) and a similar number of troglobitic species (6 and 5, respectively).

Lotic systems, besides increasing the humidity, import organic matter from the surrounding epigean environment to the inner parts of the caves. This provides food resources for the fauna (Poulson and Lavoie 2001, Souza-Silva et al. 2011a).

Caves are oligotrophic environments and the increasing resource availability allows more species to colonize and remain (Schneider et al. 2001). The amount of organic matter imported by cave streams changes depending on the season, with larger amounts during the rainy period (Souza-Silva et al. 2011a, 2012). Furthermore, during the rains many invertebrate species can be carried into the caves and, since they use organic matter as food and shelter, many species can remain throughout the year, thus increasing the local richness (Souza Silva et al. 2012).

Streams can cause disturbances in the caves, mainly during the rainy period (floods), leading to changes in the cave community (Souza-Silva et al. 2011). These disturbances are comparable to those predicted by the Flood pulse concept, initially proposed for flood plains (Junk et al. 1989). This theory predicts that the system responds in function of the range, duration, frequency and regularity of the pulses. Regular pulses (that can be the case of cave streams) lead terrestrial species to adapt to the conditions of the aquatic/terrestrial transition zones. Furthermore, regular flood pulses can prevent all of the ecological succession stages, as well as may lead to competitive exclusion.

Despite of the stress caused by flood pulses, cave streams maintain high species diversity, similar to what occurs in aquatic/terrestrial zones in flooded plains, a fact that corroborates the intermediate disturbance hypothesis (Connell 1978). It is important to emphasize that temperate and tropical areas will respond differently to the flood pulses and that the flow rate of the cave stream is also a decisive factor (Tockner et al. 2000).

The number of troglobitic species was higher in caves with puddles (Figure 4). Terrestrial invertebrates more specialized to the cave environment (troglobitic) present adaptations to live under extreme moist conditions, as, for instance, cuticular reduction that increases the tegument permeability (Culver 1982). If the permeability of the cuticle is increased, the terrestrial troglobitic are sensitive to low humidity levels, losing water quickly (Howarth 1980). Therefore, the higher richness of troglobitic species recorded in the caves with puddles poses a new question: if the presence of rivers maintains high humidity throughout the year and increase the availability of trophic resources, why do those caves did not present more terrestrial troglobitic species?

Cave streams, in spite of maintaining the high humidity and increasing the availability of resources (Souza-Silva et al. 2011a), can cause disturbances that may eventually

prevent (or make difficult) the emergence of troglobitic species. Caves that undergo violent floods usually do not present many troglobitic species (Elliott 2004). One hypothesis is that the disturbance caused by cave streams can cause constant exchange of terrestrial specimens carried by streams and, consequently, increase the genetic flow, decreasing the occurrence of speciation. This hypothesis still needs to be tested.

One of the main physiological adaptations of the troglobites is the resistance to starvation, and such organisms are more resistant to oligotrophic environments than non-troglobitic species (Huppop 2012). In caves without streams with low availability of resources the troglobitic species are certainly the best competitors. However, in caves with high availability of food resources in association with the presence of epigean species, this high availability of food may indirectly be a serious threat to troglobites (Sket 1977). Cave streams can also increase competition, especially in small caves, since more species will be brought from the external environments.

The largest number of terrestrial troglobitic species in caves with puddles indicate that these organisms are specialized to live in places with high humidity, but the disturbance caused by the presence of cave streams can eventually decrease the chances of troglobitic species to emerge. It is important to emphasize that there are exceptions, especially considering caves with large extensions. Such environments can allow distinct species to escape to areas out of the river and such big subterranean extensions certainly "filters" external fauna that could be brought during flooding pulses. However, in small caves with streams and few dry channels, terrestrial species can be severely affected and the troglobitic richness can decrease.

Beta diversity among the caves was high. The contributing factor was the replacement of species and the differences in species richness was near-zero. As we have recorded, the richness of terrestrial species is influenced by the area of the cave, size of the entrances and presence of water. These added parameters can generate strong and unique environmental filters within each cave, making it a highly heterogeneous environment. These can be some of the variables responsible for the high turnover of species between the caves. One may also consider that in tropics high values of beta diversity are expected when compared to temperate regions (Koleff et al. 2003). While in the epigean temperate regions the turnover of species suffers strong environmental influence, tropics seem to suffer more influence of spatial variations that can limit the dispersal (Myers et al. 2013). Furthermore the turnover can be higher in caves when compared to epigean areas (Cardoso 2012).

All factors here seen lead us to expect high beta diversity values. This confirms the prior predictions that high degree of micro-endemism occurs among subterranean groups (White and Culver 2012). It is important to mention that we only assessed the diversity of taxa. Considering other types of diversity such as phylogenetic and functional diversity, one would expect other still hidden patterns of diversity to emerge (Cardoso et al. 2014).

Despite the general high dissimilarity, the presence of cave streams influenced the species composition (Figure 5). This influence can be explained by two factors: *i*) the carried organic resource is similar and *ii*) the flood pulse selects the same species that are

carried into the caves (turnover). Visually, most of the organic matter carried into the caves was composed of plant debris (leaves and branches). Resources of similar origin are exploited by similar cave communities (Schneider et al. 2011), in this case, mainly detritivores. Flood pulses can carry soil species together with the organic matter (Souza-Silva et al. 2012a) and only those ones adapted to the floods and the cave environment can survive. Thus, eventually the same species have being selected in different caves.

An example is the Deus Me Livre cave. Despite it is dry during part of the year, it is subject to seasonal flooding caused by runoff during the rainy season, since its entrance is located in the bottom of a sinkhole. The fauna of this cave is more similar to the caves with streams (Figure 5), demonstrating that flood pulses caused by runoff are probably selecting the same species, supporting the previous hypothesis.

In the different Brazilian regions, the litter invertebrate fauna is composed mainly of Acari, Coleoptera, Gastropoda, Oligochaeta, Isopoda, Arachnida, Diplopoda, Chilopoda and Blattaria (e.g. Ferreira and Marques 1998, Moreira et al. 2006). These are also the main groups recorded in caves (Pinto-da-Rocha 1995, Romero 2009), what makes high the similarity between groups of cave invertebrates (especially those with streams) and soil invertebrate fauna in the Tropical region.

Another important factor is that the separation of the species according to the level of association with the cave is not always so simple (for details see Sket 2008), demanding a deep knowledge of the biology of each group, as well as of their presence (or not) in the epigean systems. Novak et al. (2012), in a study on species distribution in the cave environment, separated the groups in only two categories, troglobitic and non-troglobitic (including all other categories), precisely because of the difficulty on separating the other categories (troglophile, trogloxene and accidental).

Due to the high similarity between the litter and cave fauna and the difficulty on accurately separate which species are associated to the cave, how can we actually separate the cave fauna from the soil fauna in Neotropics? Many species carried by streams with the organic matter can contain accidental groups, although a lot of species has certainly shown to be pre-adapted to the subterranean systems. Even though these species may use the carried organic matter as shelter and food resource (Souza-Silva et al. 2012a), only with more detailed long term studies it will be possible to determine the degree of association of those species with the cave environment.

The highlight in this study is the increase in the terrestrial species richness according to metric parameters and the presence of streams, since largest entrances and water courses can influence cave colonization and detritus input. The input of organic matter by streams is important for the maintenance of cave fauna, serving as shelter and food for several species. Caves with puddles presented higher richness of terrestrial troglobites indicating that the humidity maintenance throughout the year is an important factor for the evolution and maintenance of these species. The beta diversity was high among caves, thus indicating physical and environmental heterogeneity that may be unique to each cave. Our findings highlight that big and wet caves shelter more diverse and complex terrestrial invertebrate communities, what enhances the need for conservation, management and restoration of the cave surroundings in tropical caves.

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